REMARKS

Claims 20-38, 40, 41 and 43, 44, 47-52 and 54-56 are pending in the application. Claims 20-38 are withdrawn from consideration by the Examiner as being directed to non-elected inventions. Claims 20-23, 26, 27, 30, 31, 34, 35, 38 and 56 are canceled without prejudice or disclaimer. In addition, withdrawn claims 24 and 25 are amended herein to place them in condition for rejoinder upon allowance of the product claims. Support for these amendments is found in the language of the original claims and throughout the specification, for example, at least in original claims 45 and 46; on page 7, line 25, through page 8, line 2; and on page 11, lines 15-23. It is believed that no new matter is added by these amendments and their entry and consideration are respectfully requested. In light of these amendments and the following remarks, applicants respectfully request reconsideration of this application and allowance of the pending claims to issue.

I. Rejection under 35 U.S.C. §102

The Office Action states that claims 40, 44, 47, 54, and 56 are rejected under 35 U.S.C. §102 as allegedly anticipated by Beckman et al. (U.S. Patent Application Publication No. 2003/0134307). Specifically, the Office Action states that Beckman et al. teaches a molecular beacon (MB) probe comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides at it's 5' end. The Office Action goes on to allege that because the 5' end of the MB probe would be part of the stem region, then Beckman et al. exemplifies a MB probe comprising a stem comprising one 2'-O-methyl nucleotide and one or more unmodified nucleotides. Applicants respectfully disagree with this interpretation of Beckman et al. and traverse this rejection.

Beckman et al. discusses MB probes and their degradation by the nuclease activity of DNA polymerases such as Taq (5'-3' nuclease activity) when the probe is hybridized to the target nucleic acid (Beckman et al., paragraph 0042). Specifically, Beckman et al. states "...most forms of DNA polymerase in commercial use for PCR (e.g., Taq and many common commercial variants) have a nuclease activity (e.g., a 5'-3' nuclease activity). This nuclease activity results in degradation of the MB upon binding of the MB to a target, resulting in a release of the MB label from the fluorophore. This cleavage results in signal generation, which is interpreted as MB binding, but at signal formation rates that are not as one would predict from first principles. This renders inaccurate many

quantitative aspects of real time amplicon detection with MBs." *Id.* Thus, the cleavage of the probe that Beckman et al. is concerned with is that which occurs once the probe is hybridized to the target nucleic acid.

Beckman et al. goes on to discuss the use of nuclease resistant MB probes to overcome the problem of degradation of the probe by the polymerase nuclease activity upon hybridization of the probe to the target nucleic acid (Beckman et al., paragraph 0043). MB probes made from modified nucleic acids, including 2-O methylated residues, are provided as examples of MB probes that are nuclease resistant. *Id.* The MB probes according to Beckman et al. are either made entirely of 2'-O-methyl nucleotides or comprise one or more 2'-O-methyl nucleotides at the 5' end of the MB probe (paragraph 0074). However, Beckman et al. does not clearly state which of the three 5' ends of the probe is being referred to: the first or second arms (stems) or the loop. The Office Action contends that it is the 5' end of the MB probe as a whole that is being referred to and as a result, the one or more 2'-O-methyl nucleotides would be in the stem. Applicants contend that this is an incorrect interpretation of Beckman et al. and of the nature of DNA polymerase nuclease activity.

As one of ordinary skill in the art would understand, 5' nuclease domains of DNA polymerases (e.g., Taq) "specifically recognize bifurcated ends of double stranded regions and remove single stranded 5' arms by cutting the phosphodiester bond after the first base pair of the duplex, leaving a 3' hydroxyl end." (Kaiser et al. *J. Biol. Chem.* 274: 21387-21394 (1999), page 21387. first paragraph; copy enclosed) (*See also,* Lyamichev et al. *Science* 260:778-783 (1993), page 779, first paragraph and Figure 1; and Huang et al. *Mol. Cell. Probe* 23:188-194 (2009), page 193, last sentence; copies enclosed). Thus, in the case of the MB probes, the recognition site and point of digestion would be at the 5' end of the loop region of the MB probe where it is hybridized to the target nucleic acid and where the stem region hangs off, creating a bifurcated end of a double stranded region. The resulting cleavage at this site would then be as described by Beckman et al. with the fluorophore being cleaved off from the loop region that is hybridized to the target, thus "rendering inaccurate many quantitative aspects of real time amplicon detection." (Beckman et al., paragraph 0042).

Therefore, in order to address the problem identified in Beckman et al. of reducing the nuclease degradation of MB probes that are hybridized to the target nucleic acid, Beckman et al. blocks the nuclease activity by replacing unmodified nucleotides of this 5' double stranded region with modified, 2'-O-methyl nucleotides. However, in order to block the nuclease activity, the MB probes must have the modified nucleotide at the 5' end of the loop region, where the hybridization of the probe with the target nucleic acid creates a bifurcated double stranded molecule that can be recognized by the nuclease region of the polymerase. Having a modified nucleotide at the 5' end of the stem region as suggested in the Office Action would have no blocking effect on the nuclease activity because this is not the recognition site or site of cleavage for the enzyme. Therefore, one of ordinary skill in the art would clearly understand that Beckman et al. does not teach one or more 2'-O-methyl nucleotides in the stem of a MB probe as alleged in the Office Action.

Accordingly, Beckman et al. fails to teach or suggest a MB probe comprising a stem comprising one or more nucleotides or nucleotide analogues having an affinity increasing modification, wherein said one or more nucleotides or nucleotide analogues are selected from the group consisting of a 2'-O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2'-O-methyl nucleotide as claimed in the present invention.

Therefore, applicants submit that the present invention is not anticipated by Beckman et al. and respectfully request that this rejection under 35 U.S.C. § 102 be withdrawn.

II. Rejection under 35 U.S.C. §103

The Office Action states that claims 41, 43, 48-52, and 55 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Beckman et al. as evidenced by Majlessi et al. (*Nucleic Acids Res.* 25:2224-2229 (1998)) and Tourkas et al. (*Nucleic Acids Res.* 30:5168-5174 (2002)). The Office Action states that Beckman teaches that a MB probe can comprise one or more 2'-O-methyl nucleotides or a MB probe can consist entirely of 2'-O-methyl nucleotides. The Office Action then provides a lengthy list of what Beckman does not teach and then concludes that designing probes which are equivalents to those being claimed is considered "routine experimentation especially

since MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides had already been described by Beckman" and the advantages of the 2'-O-methyl nucleotides were known. The Office Action cites Majlessi et al. and Tourkas et al. for the advantages of 2'-O-methyl nucleotides over 2' deoxynucleotides. Office Action, pages 5-6.

As stated in the Examination Guidelines for Determining Obviousness, "the Supreme Court reaffirmed the familiar framework for determining obviousness as set forth in *Graham v. John Deere Co*...." (Examination Guidelines for Determining Obviousness Under 35 U.S.C. 103 in View of the Supreme Court Decision in *KSR International Co. v. Teleflex Inc.* Federal Register Vol. 72, No. 195, 57526-57535, 57526). Hence, and as long established under that framework, to establish a *prima facie* case of obviousness, three requirements must be satisfied. First, the prior art relied upon, coupled with the knowledge generally available in the art at the time of the invention, must contain some **suggestion or incentive that would have motivated** the skilled artisan to modify a reference or to combine references. *In re Oetiker,* 24 U.S.P.Q.2d 1443, 1446 (Fed. Cir. 1992); *In re Fine*, 837 F.2d at 1074; *In re Skinner*, 2 U.S.P.Q.2d 1788, 1790 (Bd. Pat. App. & Int. 1986). Second, the proposed modification or combination of the prior art must have a **reasonable expectation of success**, determined from the vantage point of the skilled artisan at the time the invention was made. *See Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1209, 18 U.S.P.Q.2d 1016, 1023 (Fed. Cir. 1991). Third, the prior art reference or combination of references **must teach or suggest all of the limitations of the claims** . *See In re Wilson* 424 F.2d 1382, 1385, 165 U.S.P.Q. 494, 496 (CCPA 1970) ("All words in a claim must be considered in judging the patentability of that claim against the prior art"). Furthermore, as stated in *KSR Int'l Co. v. Teleflex In,* "[a] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR Int'l Co. v. Teleflex Inc.,* 550 U. S. 1, 15 (2007).

Appellants respectfully submit that the pending claims are patentable over the cited references because the Examiner has not established that any of the three requirements are satisfied. Thus, the Examiner has failed to make a *prima facie* case of obviousness.

As discussed above, Beckman et al. teaches only one or more 2'-O-methyl nucleotides in the loop region of the MB probe or MB probes that consist entirely of 2'-O-methyl nucleotides. Beckman et al. fails to teach or suggest a MB probe comprising a stem comprising one or more nucleotides or nucleotide analogues having an affinity increasing modification, wherein said one or more nucleotides or nucleotide analogues are selected from the group consisting of a 2' –O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2' –O-methyl nucleotide as claimed in the present invention.

Majlessi et al. and Tsourkas et al. fail to remedy the deficiencies of Beckman et al. Majlessi et al. does not teach MB probes but rather teaches linear probes consisting entirely of 2'-O-methyl nucleotides and Tsourkas et al. teaches MB probes consisting entirely of 2'-O-methyl nucleotides. Majlessi et al. and Tsourkas et al. compare their probes with probes consisting only of 2'-O-deoxy nucleotides (linear and MB, respectively) and find that there are advantages of using probes with all 2'-O-methyl nucleotides over those consisting entirely of 2'-deoxynucleotides. As one of ordinary skill in the art would readily understand, any advantages of 2'-O-methyl nucleotides over 2'-deoxynucleotides that these references discuss would relate only to probes that consist entirely of 2'-O-methyl nucleotides. Thus, the higher T_ms, affinities, and hybridization kinetics reported by these references can only be attributed to probes (linear or MB) comprised entirely of 2'-O-methyl nucleotides; they do not teach or suggest anything about MB probes in which only some of the 2'-deoxynucleotides are replaced with 2'-O-methyl nucleotides.

The Office Action concludes that although Majlessi et al. and Tourkas et al. compare probes consisting of 2'-O-methyl oligoribonucleotides with probes consisting of 2'-deoxy oligoribonucleotides, one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2'-deoxy nucleotides would also be useful. Office Action, page 7. The Office Action goes on to state that the prior art is replete with guidance and information necessary to permit the ordinary artisan to design MB probes that have better stability, do not open spontaneously and are more sensitive to polymorphisms. *Id.* However, no support is provided for either of these conclusory statements.

As stated in KSR, rejections based on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness (550 U.S. __, 82 USPQ2d 1385, 1396 (2007); see also Examination Guidelines for Determining Obviousness Under 35 U.S.C 103 in View of the Supreme Court Decision in *KSR International Co. v. Teleflex Inc.* Federal Register Vol. 72, No. 195,57526-57535, 57528).

The present Office Action provides no evidence to support the allegation that one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2'-deoxy nucleotides would also be useful or the allegation that the prior art is replete with guidance and information to permit the ordinary artisan to design MB probes that have better stability, do not open spontaneously and are more sensitive to polymorphisms. Certainly, none of the cited references provide such support.

In particular, Majlessi et al. compares linear probes of different lengths and does not teach or suggest MB probes or solutions to problems associated with the premature opening, stability or specificity of such probes (some of these problems, such as premature opening, do not even apply to linear probes). Further, as discussed above, Majlessi et al. does not teach or suggest probes having a combination of 2'-O-methyl nucleotides and 2'-deoxy nucleotides for any purpose, much less to improve sensitivity to polymorphisms in the target nucleic acid or to prevent the premature opening of a MB probe as is claimed in the present invention. Thus, Majlessi et al. fails to provide any teaching or suggestion that would motivate one of skill in the art to combine Majlessi et al. with Beckman et al. so as to produce a MB probe comprising a stem comprising one or more nucleotides or nucleotide analogues having an affinity increasing modification, wherein said one or more nucleotides or nucleotide analogues are selected from the group consisting of a 2' –O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2' –O-methyl nucleotide as claimed herein nor would such a combination provide any reasonable expectation of success in achieving the presently claimed invention.

Tsourkas et al. describes MB probes consisting entirely of 2'-O-methyl nucleotides or probes consisting entirely of 2' deoxy nucleotides. Tsourkas et al. teaches that MB probes consisting of 2'-O-methyl nucleotides have a reduced ability to discriminate between the wild-type target and the mutant target nucleic acids (Tsourkas et al., page 5169, first column, first full paragraph. last sentence). Thus, one of ordinary skill in the art reading Beckman et al. as evidenced by Tsourkas et al. and desiring to increase the sensitivity of a probe to detect polymorphisms would be lead away from substituting unmodified nucleotides with modified because Tsourkas et al. teaches that this would in fact reduce sensitivity.

Further, one of ordinary skill in the art reading Beckman et al. even in combination with Tsourkas et al., and interested in improving a MB probe for diagnostic assays would not consider, for example, combining an unmodified nucleotide with a modified nucleotide in a base pair in the stem as claimed herein because such a skilled person would understand from the prior art that this would decrease the stability of the MB probe. Neither Tsourkas et al. nor Beckman et al. provide any teaching or suggestion for such a modification. In fact, Tsourkas et al. teaches away from such a MB probe in the statement that "...2'-O-methyl molecular beacons form a more stable stem-loop structure because of the 2'-O-methyl/2'-O-methyl interactions." (Tsourkas et al., page 5173, first column, last sentence). Accordingly, one of ordinary skill in the art having read Beckman et al. and Tsourkas et al. and even being interested in designing a probe with better stability that does not open spontaneously would not design a probe having the combination of an unmodified nucleotide with a modified nucleotide in a base pair in the stem as claimed in the present invention.

Contrary to the teaching of Tsourkas et al., applicants have surprisingly discovered that the designing of a MB probe having better stability that does not open spontaneously depends not only on the presence of nucleotide analogues in the stem but also on the number of nucleotide analogues, their position in the stem or loop of the MB probe and the sequence of the stem or loop of the MB probe. See for example, Table 6 of Example 4 of the present specification, which shows that MB probes consisting entirely of base pairs having only the same type of nucleotide (unmodified or 2'-O-methyl nucleotides) results in increased levels of spontaneous opening of the probe. Notably, the

MB4 probe having all modified nucleotides has a greater percentage of spontaneous opening (IBL-Increase of Baseline) than reference MB which is comprised entirely of unmodified nucleotides. The MB4 probe also has a greater percentage of spontaneous opening as compared to MB probes comprising a combination of unmodified and modified nucleotides. Furthermore, as shown by the other MB probes provided in Table 6, the position and number of nucleotide analogues in the probe are clearly shown to affect rates of spontaneous opening. None of the cited art teaches or suggests that the content and placement of the modified nucleotides in an MB probe would play an important role in the functional features of the MB probe.

Thus, the combination of Beckman et al. and Tsourkas et al. teaches away from the claimed invention which provides a molecular beacon probe comprising a stem and a loop, wherein said loop comprises: one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and one or more unmodified nucleotides; and said stem comprises: one or more 2'-O-methyl nucleotides, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2'-O-methyl nucleotide, wherein the sensitivity of said probe to polymorphisms in the target nucleic acid sequence is lowered as compared to a molecular beacon probe without said loop and wherein the spontaneous opening of the probe in the presence of contaminants present in an amplification enzyme mixture comprising said molecular beacon probe is lowered as compared to a molecular beacon probe without said.

Accordingly, in view of the foregoing, applicants respectfully submit that Beckman et al., Majlessi et al. and Tourkas et al., alone or in combination, fail to teach or suggest the presently claimed invention, and thus, request the withdrawal of this rejection.

IV. Rejoinder of method claims

Applicants further specifically request that the method claims 24, 25, 28, 29, 32, 33, 36, and 37 be rejoined and examined in the present application, once a determination is made that the composition claims under examination are allowable. Specifically, if composition claims 40, 41, 43, 44, 47-52, and/or 54-55 are found to be allowable, applicants request that the Examiner review and examine claims 24, 25, 28, 29, 32, 33, 36, and/or 37, which recite all of the limitations of the

composition claims, according to the practice of rejoinder as set forth in Section 821.04 of the MPEP. In particular, it is stated therein that if a product claim is elected in a restriction and then found allowable, withdrawn process claims that depend from or otherwise include all the limitations of the allowable product claim are to be rejoined.

The points and concerns raised in the Action having been addressed in full herein, it is respectfully submitted that this application is in condition for allowance, which action is respectfully requested. Should there be any remaining concerns, the Examiner is encouraged to contact the undersigned attorney by telephone to expedite the prosecution of this application.

The Commissioner is authorized to charge Deposit Account No. 50-0220 in the amount of $810.00 for a Request for Continued Examination. This amount is believed to be correct. However, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-0220.

Respectfully submitted,

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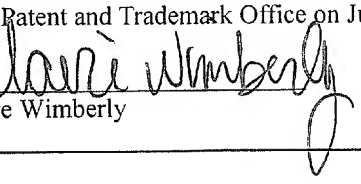

Claire Wimberly